Cellular plasticity: a mechanism for homeostasis in the kidney

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Cellular plasticity: A mechanism for homeostasis in the kidney

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Abstract

Cellular plasticity is a topical subject with interest spanning a wide range of fields from developmental biology to regenerative medicine. Even the nomenclature is a subject of debate, and the underlying mechanisms are still under investigation. On top of injury repair, cell plasticity is a constant physiological process in adult organisms and tissues, in response to homeostatic challenges. In this review we discuss two examples of plasticity for the maintenance of homeostasis in the renal system—namely the renin-producing juxtaglomerular cells (JG cells) and cortical collecting duct (CCD) cells. JG cells show plasticity through recruitment mechanisms, answering the demand for an increase in renin production. In the CCD, cells appear to have the ability to transdifferentiate between principal and intercalated cells to help maintain the highly regulated solute transport levels of that segment. These two cases highlight the complexity of plasticity processes and the role they can play in the kidney.

KEYWORDS

collecting duct, JG cells, kidney, plasticity, renin

1 | INTRODUCTION

Many conventional biological research techniques can only offer snapshots of cellular mechanisms and behaviours; it may be why a refocus on the inherent "plasticity" of organisms and physiological processes is timely. While adult tissues are known to be relatively stable and mostly composed of cells in a differentiated state, a growing body of evidence shows an environment in constant flux, helping to redefine what "stable" really means in a living organism. Recent techniques such as single cell RNA-sequencing (scRNASeq) show that differentiated cells of the same type can express different levels of specific markers. Pseudo-time analysis of scRNA-Seq data can provide insights into the relationships between populations of cells and their progression from one state, or cell type, to another.[1] Such analyses have recently brought the capacity for cell plasticity into sharp focus. However, the definition of "plasticity" is debated, and used quite flexibly in different areas of research, as illustrated by the commentary of a workshop on nomenclature by Mills et al[2] The authors point out that a common language is still needed in the scientific community, since different phenomena are described under the umbrella of plasticity.

Regardless of the specific nomenclature debate, cell plasticity is commonly and broadly defined as the ability of a cell to convert into a different cell type. Several types of plasticity have been described and are illustrated in Figure 1. More specifically, plasticity has been defined to happen either by dedifferentiation, transdifferentiation, or transdetermination.[3-6] Dedifferentiation is the process through which a differentiated cell will revert to a stem or progenitor-like cell type. Transdifferentiation is the process through which a cell changes from one differentiated cell type to another. Transdetermination describes the conversion of a specific

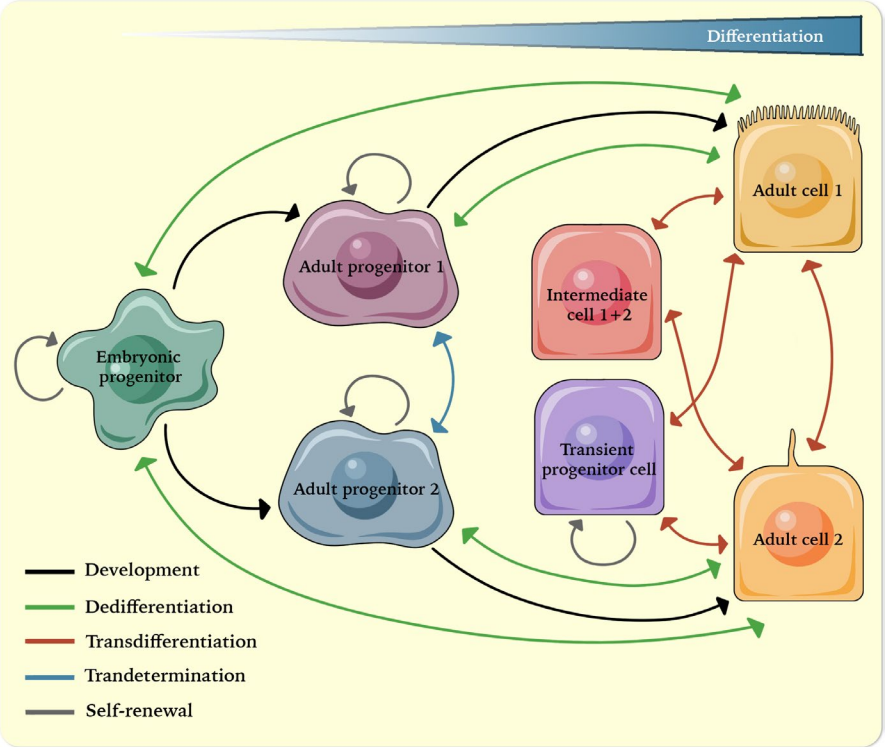

FIGURE 1 The different definitions of cell plasticity. Plasticity phenomena are shown in contrast to the conventional unidirectional path taken by cells during development, from embryonic progenitor to differentiated adult cell (in black). Plasticity through dedifferentiation (in green) refers to adult cells reversing to the adult, or embryonic, progenitor state. Transdifferentiation (in red) shows the switch between two adult cell types either directly or through a transient progenitor or intermediate cell type. Transdetermination (in blue) is the switch between two types of progenitor cells

lineage progenitor cell into an alternative lineage progenitor cell.[7] A recent study also introduces the concept of paligenosis which can be considered a subtype of dedifferentiation mechanism. Typically observed in injury response mechanisms, paligenosis describes the process of converting a differentiated cell into a regenerative and proliferative cell type using evolutionary conserved mechanisms characterized by mTORC signalling-induced cell-cycle re-entry.[8-10]

To complicate the picture, each type of plasticity possesses an array of underlying causes and mechanisms, which may be specific to cell location and function in adult tissues. Injury response is the most obvious trigger of cell plasticity, and uses both de-differentiation and transdifferentiation mechanisms, such as in limb regeneration in amphibians or macrophages switching cell types respectively.[11,12] However, another physiological cause of cell plasticity is starting to emerge—maintaining homeostasis in adult tissues. In the kidney in particular, plasticity is under intense investigation because of the essential role of the organ to maintain body volume, fluid osmolarity, acid-base balance and the links between kidney disease and malfunction of blood pressure control. Whilst cell plasticity is a whole-body phenomenon, it appears particularly complex in the kidney because of the complexity of the organ itself, possessing dozens of different cell types forming a hormonally regulated filtration and reabsorption system.[13] To illustrate the importance of cell plasticity for maintaining homeostasis this review will focus on two examples of renal cell plasticity: collecting duct cells, and renin-expressing cells (juxtaglomerular cells or JG cells). Both directly contribute to the Renin-Angiotensin-Aldosterone System (RAAS) which regulates blood pressure, fluid and electrolyte balance and systemic vascular resistance.[14]

2 | EVIDENCE FOR PLASTICITY IN THE KIDNEY

Studies of effect of the environment on cell physiology in the kidney show a constant adaptation of cellular structures through mechanical and chemical stimuli. For example, cell phenotype has been shown to change in response to altered tubular fluid composition or flow, and proteinuria following glomerular damage leads to tubulointerstitial inflammation and fibrosis via protein binding at the apical pole of proximal tubular cells.[15] Flow is used in in vitro research and kidney on chip technologies because it drives cell differentiation and improves cell function: IMCD (inner medullary collecting duct) rat primary cells have shown better viability under oxidative stress conditions when subjected to flow, and show altered cytoskeleton organization, increased tight junctions and different adhesion sites.[16]

Several studies report that progenitor markers expressed during kidney development are also expressed in adult cells, suggesting paligenosis or a reserve of progenitor-like cells. During early development, kidney structures derive from two main sources: the ureteric bud and the mesenchyme, which give rise to different lineages expressing lineage-specific markers.[17] During maturation of the ureteric bud, the "neck" of the structure possesses a transitional precursor cell type.[18] Some cells of the adult kidney retain characteristics of the

developmental kidney as seen in label retaining tubular cells that can regenerate tubular structures.[19]

Cellular modification after kidney injury is an active area of research, even though the mechanisms are not fully understood. The consensus is that renal structures possess relatively efficient repair mechanisms after acute injury, but less so during chronic kidney disease which represents a continuous stress on the cells. The kidney tubules have been shown to possess reserves of progenitor-like cells in the S3 segment as well as stochastic dedifferentiation mechanisms, especially in the proximal tubule, that can help repopulate the area in case of injury.[20-23] Progenitor cells have also been identified in the Bowman's capsule and taken together, they represent less than 2% of the total number of cells in the kidney.[24] On the other hand, deleterious mechanisms also exist: podocytes and parietal epithelial cells (PECs) can transdifferentiate into macrophagic cells and myofibroblasts, increasing fibrosis during kidney disease during a phenomenon known as epithelial-to-mesenchymal transition (EMT).[25] EMT describes the transition from polarized epithelial cells to mesenchymal cells, and whilst generally considered a deleterious process in the adult kidney, it is receiving attention as a potential repair mechanism involving dedifferentiation, proliferation and then re-differentiation.[26,27] Studies investigating EMT have also shown the presence of "hybrid" cells, expressing both markers of progenitor and differentiated cells.[28] In the collecting duct, cells under stress and undergoing EMT have been shown to express Pax2, a factor typically associated with nephrogenesis. Pax2 expression protected cells from apoptosis in a model of renal fibrosis. While often observed during renal injury, some cells spontaneously display these characteristics when cultured, suggesting a capacity for plasticity under more normal physiological conditions.

3 | MECHANISMS OF PLASTICITY OF JG CELLS

One of the key hormones effecting blood pressure and electrolyte control is renin, which is produced and secreted by a small number of juxtaglomerular cells (JGCs) that make up approximately 0.01% of the adult kidney. Renin-precursor cells (RPC) originate from mesenchymal cells in the metanephric kidney. Their appearance broadly follows the developing vasculature of the kidney—firstly in the arcuate arteries, then the interlobar arteries and finally the afferent arterioles.[29] RPCs are also observed in the glomerular mesangium and interstitium,[30] reflecting their derivation from $Foxd1^+$ stromal cells and their subsequent differentiation into mesangial cells and interstitial pericytes.[31] During kidney development, RPCs co-express smooth muscle cell markers, such as αSMA, suggesting that the newly formed vessels may be stabilized by RPCs, which are dotted along the arteries giving a striped appearance.[29] As kidney development proceeds, renin expression regresses from proximal to distal parts of the arterial tree until finally it is retained only in the JGCs of the afferent arterioles as they enter the glomeruli. JG cells retain myofilaments and a pericyte-like morphology as they encircle the afferent arteriole. They are often described as modified, granular myoepithelioid cells.

JG cells store renin in dense core secretory granules, which are modified lysosomes. Renin is produced and released from the granules in response to complex signals.[32] These include tubulo-glomerular feedback from the NaCl-sensing Macula Densa, mediated through NO and prostaglandins[33] and sympathetic nervous stimulation mediated via β-adrenergic receptors, and leading to stimulation of G protein-coupled receptors, adenyl cyclase and the cAMP second messenger.[34] Unusually for secretory cells, renin release is augmented by a reduction in intracellular calcium—the so-called calcium paradox. Cytosolic calcium has been shown to inhibit adenylate cyclases V and VI and cause the degradation of cAMP via calmodulin-activated phosphodiesterase1C.[35,36] Transcriptome analysis of the JGC reveals the expression of multiple smooth muscle-associated genes in addition to high levels of renin[37] and other markers of the JGC, such as Akr1b7. These include smooth muscle myosin heavy chain (SM-MHC, Myh11), calponin (Cnn1) and regulators of G protein signalling (Rgs5, Rgs2). This suggests that JGCs retain both contractile and endocrine functions in order to effect control of blood pressure and fluid-electrolyte homeostasis.[37]

Reductions in BP or extracellular fluid volume, with or without hyponatremia, lead to release of renin. Under a significant or chronic threat to homeostasis, such as hypotension or dehydration, SMCs revert to a renin-expressing phenotype that they exhibited during renovascular development, a process commonly referred to as "JG cell recruitment". The recruited cells are derived from the same RPCs that previously lined the afferent arteriole (and if required, interlobar arteries). They are transformed into their pre-differentiated state, taking on the properties of their precursor cell type to contribute to overall renin production and temporarily add to the small pool of renin producing cells. This was demonstrated by using dual fluorescent probes to distinguish between RPCs (cyan fluorescent protein; CFP) and JGCs (yellow fluorescent protein; YFP).[38,39] In response to homeostatic stress, the subset of VSMCs derived from RPCs (cyan) undergo a process of dedifferentiation to regain their renin expressing properties (YFP). The RPC-specific reporter is expressed in a striped pattern, typical of developmental-expressed renin. Recruited cells in rat micro-vessels have been shown to exhibit the calcium paradox,[40] while in mice, strong co-regulation of renin and lysosomal integral membrane protein 2 (Limp2) has been demonstrated in recruited cells.[41] Under extreme conditions, interstitial peritubular pericytes and mesangial cells also develop the capacity to synthesize and

release renin,[42] enabling them to contribute to the re-establishment of homeostasis. It may be beneficial for cells to be recruited or de-differentiated in order to produce renin, as opposed to cell proliferation or cell migration, as recruitment may be more energy efficient in times of challenge to homeostasis. It also suggests that the cells derived from RPCs retain a "genome memory" of their progenitors. A key element of plasticity is the ability for such morphological changes to be reversed, and upon the return of homeostasis the transformed cells lose their acquired renin-expressing qualities and morphology and regain their original phenotype.

JG cells in primary culture rapidly lose their ability to store renin in granules and down regulate renin expression.[43] Cells of the renin lineage marked with CFP, however, were shown to retain the ability to re-express renin (marked by expression of YFP), under appropriate stimulation with forskolin and cAMP analogues.[38] The cAMP-induced re-expression of renin is brought about by the recruitment of activated CREB, and its co-activators p300 and CREB-binding protein (CBP) (see Figure 2). These histone acetyl transferases have been shown to relax the chromatin upstream of the renin gene, allowing access to transcription factors such as Creb1. Conditional deletion of both p300 and CBP in renin cells of mice prevented recruitment of RPCs, when they were subjected to homeostatic challenge. These mice also showed abnormal arteriolar development, indicating the important role that RPCs play in maintenance of kidney vasculature.[44] The importance of the cAMP pathway was demonstrated by the JGC-specific deletion of G protein Gsα, which also reduced the number of renin cells during development and led to renal failure.[45]

3.1 | Cell-cell communication

Cell fate decisions often include cell-cell communication, as typified by the Notch pathway. A number of Notch pathway genes, including Notch3 and Hey1 were identified in the JGC.[37] The final transcription factor of the Notch pathway, RBP-J, was shown to affect renin cell number and renin cell recruitment since both were significantly reduced by JGC-specific deletion of RBP-J.[46] Similarly, mutation of four core nucleotides in the RBP-J binding site of the renin promoter was sufficient to suppress GFP expression, and recruitment of renin cells on sodium depletion or captopril treatment.[47] Since RBP-J also controls the expression of smooth muscle genes and their master regulators, this suggests that the Notch pathway is intimately involved in renin cell plasticity and recruitment of smooth muscle cells. Likewise, the observation that connexin-40, a transmembrane protein involved in gap junctions, is highly expressed in JGCs[37] and that its knockout leads to the loss of JGC and appearance of RPCs in the periglomerular interstitium[48] suggests that cell-cell communication is also essential for JGC positioning.

3.2 | MicroRNAs

MicroRNAs are known to repress translation by targeting specific mRNAs and are thus able to affect cell fate. Conditional deletion of Dicer, the RNAse III endonuclease responsible for maturation of microRNAs, in RPCs led to a reduction in

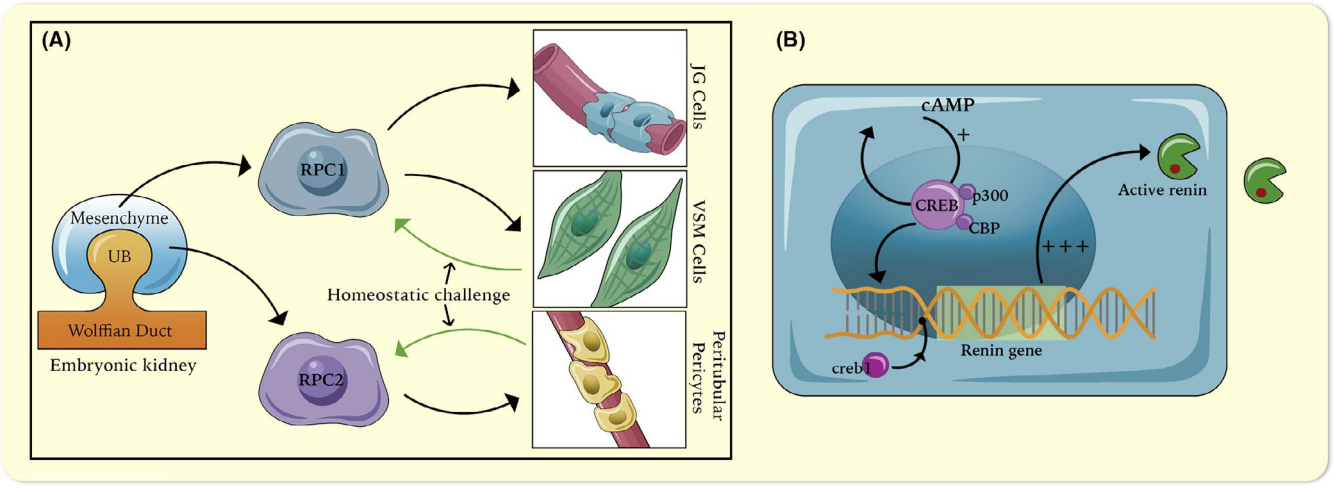

FIGURE 2 Mechanisms of plasticity in renin producing cells. A, Renin precursor cells (RPCs) originate from the metanephric mesenchyme of the embryonic kidney, distinct from the ureteric bud (UB) which will develop into the collecting system of the kidney. RPC1 are situated around the afferent arteriole of the glomerulus and will differentiate into JG cells (renin producing) and vascular smooth muscle cells (VSMCs). RPC2 are situated in the mesangium and interstitium and will differentiate into peritubular pericytes. Under homeostatic challenges, both VSMCs and peritubular pericytes are able to dedifferentiate into RPC1 and RPC2 respectively to release renin again. B, The cAMP pathway inducing the re-expression of renin in culture. CREB and co-activators lead to the relaxation of chromatin upstream of the renin gene, allowing for transcription factors including creb1 to bind and induce renin expression

JGCs, renin levels and blood pressure,[49] suggesting that microRNAs may be important in maintaining JGCs. Medrano et al[50] then identified two microRNAs, miR-330 and miR125b-5p, which were important for recruitment. The former microRNA inhibits smooth muscle phenotype in JGCs while the latter induces the contractile phenotype in VSMCs and is repressed during recruitment.

3.3 | Mesenchymal to epithelial transformation

Podocytes are terminally differentiated cells, which are unable to proliferate or self-renew.[51] Replacement of damaged podocytes therefore relies on local kidney progenitors, of which RPCs and PECs are candidates. Cells of renin lineage have been shown to undergo mesenchymal to epithelial (MET) transformation, with an increase in Wilm's tumour suppressor1 (Wt1) gene expression and concomitant reduction in SMC markers and expression of new epithelial markers such as E-cadherin.[52] Animals specifically lacking WT1 in RPCs showed reduced proliferation and migration of the RPCs and worse glomerular disease than controls. Transdifferentiation of RPCs to podocytes was independently confirmed using dual lineage tracing by Eng et al.[53] Using multicolour reporters and intravital imaging Kaverina et al[54] showed that, following abrupt podocyte depletion, RPC reporters were subsequently identified within a subset of glomeruli, which co-expressed either podocyte or PEC markers. Migration of a JGC to the Bowman's capsule was also directly visualized. Starke et al demonstrated that depletion of mesangial cells from the glomerular tuft leads to replenishment from extra-glomerular RPCs.[55] They used transgenic mice to demonstrate the increase in green fluorescent RPCs in glomerular tufts during the repair process. Interestingly, LacZ-expressing reporter RPCs further demonstrated that the newly formed mesangial cells no longer expressed renin.

4 | MECHANISMS FOR CELL PLASTICITY IN THE COLLECTING DUCT

The main function of the collecting duct is to receive the filtrate from the nephron and reabsorb water and sodium, regulated in part by the RAAS.[14] Collecting ducts are lined by simple cuboidal epithelium, merge as they go through the medulla, and lead to the ureter. The cortical collecting duct (CCD) plays a central role in the final volume and concentration of urine: two-thirds of the hypo-osmotic fluid entering the collecting duct is reabsorbed in that segment.[56] The collecting ducts are composed of principal cells (PCs) and α and β intercalated cells (ICs). PCs and ICs possess key functional and morphological differences and cooperate to regulate acid-base and volume homeostasis. PCs reabsorb water and sodium through aquaporin 2 (Aqp2) and epithelial sodium channels (ENaC) respectively,[57] are responsible for K^+ excretion and express several physiologically important genes including HSD11b2.[58] ENaC is a direct target of the hormone aldosterone that binds with high affinity and results in an increase in Na^+ reabsoption.[59] The main function of ICs is the regulation of urinary pH through the secretion of protons (H^+) and bicarbonate (HCO_3^-).[60] (See Figure 3 for cell type characteristics).

Interest in plasticity of collecting duct cells is not new. Schwartz et al[61] showed that ICs can change from β- to α-type when subjected to acidic culture medium, which further demonstrates a certain cell plasticity driven by environmental cues. Earlier work showed the possibility for β-IC to give rise to both α-IC and PCs.[62] More recently, Wu et al showed ICs originating from PCs through lineage tracing of $Aqp2^+$ cells.[63] Recent work from Park et al[64]confirms plasticity between PCs and ICs with fluorescent lineage tracing, showing PCs originating from IC-marker tagged cells and *vice versa*. Knock-out of the histone H3 K79 methyltransferase Dot1l led to a decreased number of PCs and increased number of both α and β-ICs,[65] and Dot1l has also been reported to regulate the expression of PCs markers such as sodium channel ENaC.[66] Environmental factors have been shown to influence cell type in the CCD. Gao et al showed deletion of hensin/DMBT1, an extracellular matrix component, blocks the conversion of β to α-IC.[67] Treatment of adult rats with acetazolamide, a carbonic anhydrase II (CAII, IC marker) inhibitor, led to the remodelling of the collecting duct cells, with an increase in α-IC prevalence and decrease in PCs and β-ICs.[68] Similarly, lithium treatment not only downregulated the expression of PCs markers involved in sodium and water reabsorption in the CCD, but also resulted in an increased ratio of ICs to PCs.[69] Both remodelling phenomena were thought to be a corrective mechanism in response to the metabolic acidosis resulting from treatment.

4.1 | Hybrid/transitional cells

In 1999, Kim et al were first to identify a non α- non β-IC type,[70] suggesting a reserve of "intermediate" ICs able to transform into either α or β subtypes. Since then, the observation has extended from IC types to include PCs. Several studies have identified cells expressing markers of both cell types, initially using immunohistochemistry. Recent development of techniques such as single-cell RNA sequencing (scRNASeq) have more explicitly identified reserves of "intermediate" cell types, found only in the collecting duct.[64,71] More specifically, single cell sequencing of primary CCD cells by Chen et al found a subset of "hybrids" expressing

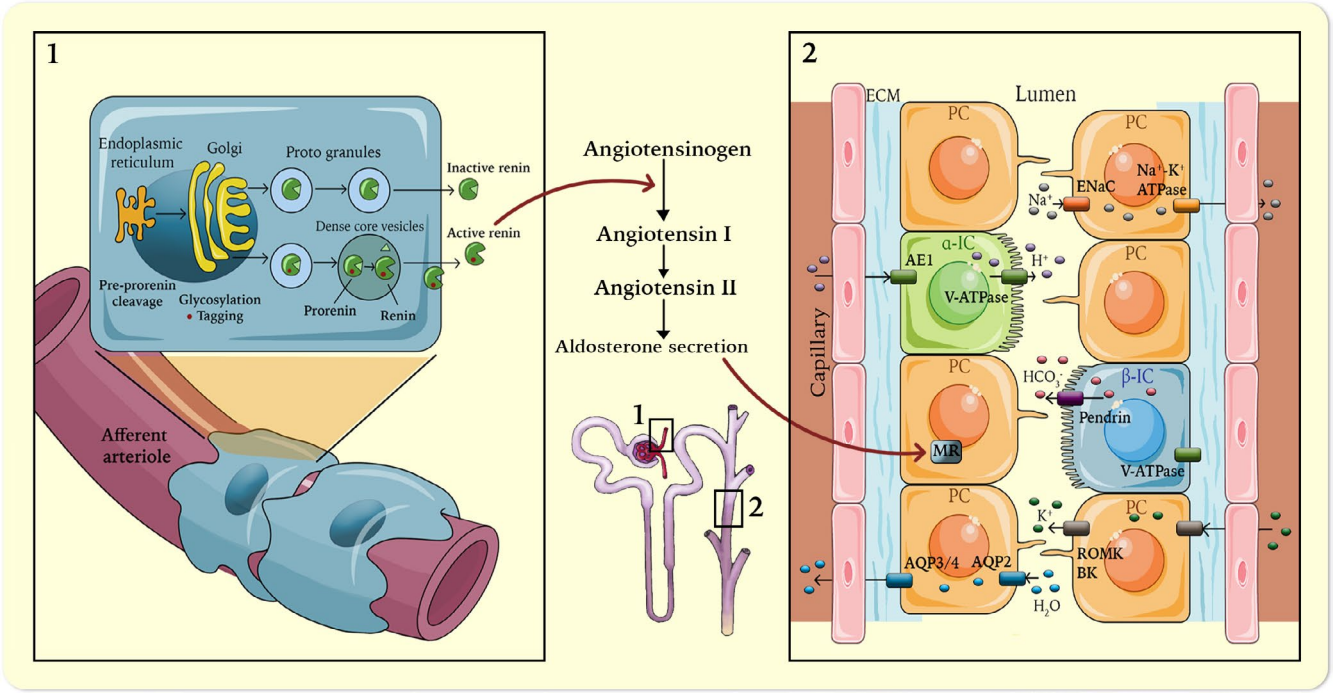

FIGURE 3 The physiology and anatomy of JG cells and the collecting duct in the RAAS system. In the centre is pictured a nephron functional unit of the kidney. Sodium and water reabsorption are directly regulated in the collecting duct by the hormone aldosterone, produced in the outer section of the adrenal cortex of the adrenal gland. Renin, produced by granulated JG cells (box 1), is necessary for the production of aldosterone through an upstream mechanism (summarized in the centre). Pre-prorenin is cleaved into prorenin in the endoplasmic reticulum. Box 2, the highly polarized collecting duct epithelium possesses two main cell types, principal cells (PCs) and intercalated cells (ICs) α and β, with distinct physiological functions. The main functions of PCs are the reabsorption of a regulated amount of sodium $(Na)^+$, reabsorption of water and potassium (K^+) regulation. The main functions of ICs are acid-base balance through the transport of H^+ by α-ICs and of bicarbonate (HCO_3^-) by β-ICs

both Aqp2 (PC marker) and AE1 or Pendrin (IC marker), although as the authors mention, the physiological role of intermediate cells remains to be determined. The work by Park et al[64] also identified "hybrid" cells in the CCD, although with different markers (Atp6v1g3 and Atp6v1b1 for ICs). These cells possess specific markers identifying them as a new cell type, and had a greater overlap in their gene expression profiles with ICs than PCs. Pseudotime analysis[1] suggested an intermediate status, which seemed stable (non-proliferative, non-injury cells). Clues to explain the presence of these intermediate cells include the cell specific markers: Parm1, Sec23b, Syt7. Parm1 has been associated with early urogenital development,[72] while the role of Sec23b in the kidney is unknown but is associated in general with protein export from the endoplasmic reticulum.[73] Syt7, a calcium-sensing protein, is mostly expressed in endocrine cells.[74]

In vitro models of collecting duct cells such as $mCCD_{cl1}$ cells (self-immortalized mouse CCD cell line[75]) have shown transitional phenotype too, and capacity for plasticity retained through single cell cloning.[76] Interestingly, $mCCD_{cl1}$ cells still exhibit the expected functions of PCs such as amiloride-sensitive sodium transport,[75] suggesting that hybrid cells are still capable of physiological function, but that observation has not been confirmed in vivo.

Whilst the available evidence indicates direct, or indirect, plasticity between PC and IC cells, we cannot exclude the possibility that an unidentified population of progenitor cells may exist and play a role in determining PC/IC cell ratio. If such cells exist then they may represent an intermediate between ureteric bud cells and hybrid/transition cells of the mature collecting duct, however this remains hypothetical.

4.2 | The Notch pathway in CCD cell fate determination

The Notch pathway is associated with the sequential emergence of cell lineages from progenitor cells in general,[77] and with nephrogenesis in particular.[78] In the adult collecting duct, multiple factors involved in the Notch pathway have been linked to cell plasticity. The consensus is that blocking or downregulating the Notch pathway during kidney development and in adult collecting ducts results in a decreased number of PCs, and increased number of ICs. A summary of what is known about the Notch pathway for CCD plasticity is shown on Figure 4. The ratio of principal to ICs, typically 70:30 in mice,[79] was influenced in several studies by knock-out of factors required in Notch

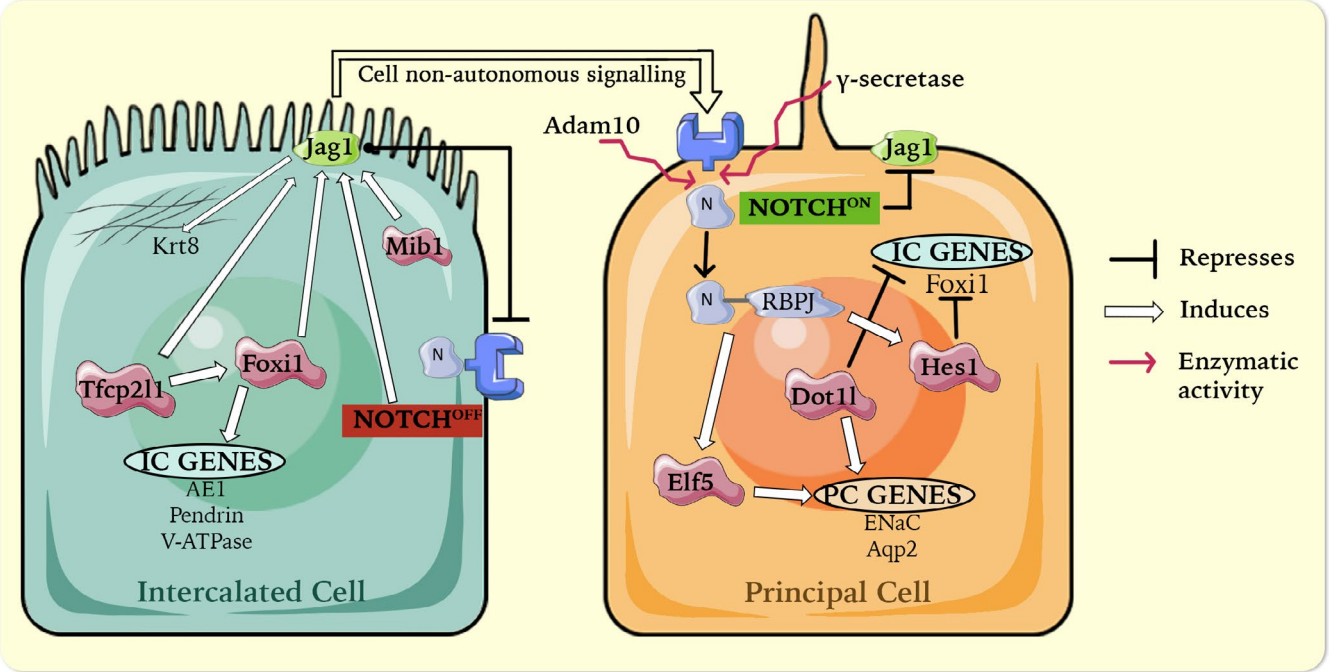

FIGURE 4 Summary of known factors involved in CCD cell fate determination through the Notch pathway. Intercalated cells are stuck in a Notch^{Off} feedback loop, because of the expression of Notch ligand Jag1 in the same cell. On the other hand, principal cells are in a Notch^{On} configuration, with the cleavage of the Notch intracellular domain resulting in a cascade of effects inducing principal cell genes expression and repressing intercalated cell gene expression. External factors such as lithium treatment, affect the Notch pathway by repressing Hes1 which leads to a reduced number of principal cells and increased number of intercalated cells. CCD, cortical collecting duct

signalling such as the transcription factor Adam10[79] and the E3 ubiquitin ligase Mib1.[80] In these studies, the deletion of floxed alleles of Adam 10 and Mib1 via genetic crosses with Aqp2-cre or Hoxb7-cre mice respectively resulted in a reduced number of PCs and a corresponding increase in the number of ICs. This shifting ratio was not accompanied by cell death or a significant shift in cell number, strongly suggesting a switch between cell types. Adam10 cleaves the membrane-bound precursor of TNF-alpha to its mature soluble form; it controls the proteolytic processing of Notch and mediates lateral inhibition mechanisms during development.[81] Mib1 has been shown to be necessary to the activation of Jag1, a Notch ligand produced in ICs, and thus of Notch signalling in mammals.[82]

Another transcription factor involved in the Notch pathway, Tfcp2l1 has been shown to coordinate the development of the kidney collecting ducts intercalated and PCs.[83] More specifically, Tfcp2l1 binds the promoter of Jag1 and Hes1, regulating the expression of Jag1 and subsequent Notch signalling in the collecting ducts.[84] Required for normal duct development in the salivary gland and kidney, Tfcp2l1 regulates the expression of IC genes including V-ATPase B1 and D2, Slc4a1, Aqp6 and transcription factor Foxi1. In return, IC specific genes are thought to bind Tfcp2l1, Hes1 and Foxi1, suggesting a functional feedback loop. Elf5, another target of the Notch pathway, is the only factor apart from Dot1l mentioned earlier (which positively regulates ENaC[66]) described to have a direct positive influence on PC specific marker expression. It contributes in particular to Aqp2 and Avpr2 expression.[85]

4.3 | Hybrid cells in a tissue regulated by Notch signalling

Involvement of the Notch pathway in cell type determination in the collecting duct was suspected because of the "salt and pepper" or "rosette-like" principal/IC patterning along the duct. Previous studies described similar patterning of cells in other tissues because of the lateral-inhibition paradigm of Notch signalling.[86-88] In the collecting duct this pattern appears to be driven primarily through expression and secretion of the Notch ligand Jag1 by ICs. As discussed above, Jag1 secretion initiates Notch signalling in PCs but inhibits Notch signalling in ICs.[84] In the healthy adult kidney, cells are typically locked in a Notch^{on} or Notch^{off} feedback loop (see Figure 4), determining their expression of PC or IC specific markers. Notch is located on the basolateral membrane in ICs, which may present a physical barrier to the binding of Jag1. Hybrid cells found in the collecting duct may therefore be out of that feedback loop. Clues to understanding the mechanisms behind the regulation of the Notch paradigm, and the presence of hybrid cells, include a study that produced a collecting duct entirely composed of

hybrid cells by knocking out Foxi1.[89] Foxi1 is a forkhead gene that has previously shown links to pendrin regulation in the adult inner ear[90] and been established as an important factor for collecting duct cell identity during kidney development.[91] In this study, Foxi1$^{-/-}$ mutant mice had collecting duct cells expressing both CAII and AQP2, intercalated and PC markers respectively. Foxi1 was shown to be an important regulator of IC markers and Jag1 expression: Foxi1$^{-/-}$ cells lacked V-ATPase B1, Pendrin, AE1 and AE4. In the adult, this establishes Foxi1 as a "blocker" of CCD cell plasticity. Hes1 has recently been shown to directly repress the expression of Foxi1 in PCs, preventing the IC "program" from being expressed.[92] mCCD$_{cl1}$ cells mentioned previously have very low, or non-existent, expression of Foxi1. They also lack pendrin, AE1 and AE4, but express V-ATPase B1. The recent study examining the role of transcription factor Tfcp2l1 in CCD cell patterning[84] found that during development, Tfcp2l1 induces the creation of similar double-positive progenitor cells (ie expressing both principal and IC markers) before playing its role as an IC regulator.

5 | DISCUSSION

5.1 | Questions yet to be answered regarding cell plasticity

The scale of cell plasticity in healthy tissues has only recently become apparent. Many techniques used to study "healthy" and pathological states, or the effect of a gene knock-out, typically only reveal a "snapshot" of cell functions, whilst plasticity is, by nature, a time-sensitive phenomenon. Maintenance of homeostasis includes negative feedback mechanisms and hormonal regulation, which may also involve cell plasticity. Circumstantial evidence such as the constitutive ability for cell plasticity in response to injury, or the regulation of acidity in the CCD by changing α-ICs to β-ICs, suggest constant adaptation of healthy tissues. On the other hand, the extensive work by Park et al which identified stable transitional cells between principal and ICs suggest that the rate of transition between the two cell types is low in healthy tissues. Both rate of change and number of transition cells may increase during disease states such as CKD,[64] based on pseudotime analysis of the sequencing data.

The Notch pathway is a big player in kidney development, both in the early collecting ducts[93] and the early nephrons,[94] but it is also intimately involved in JG cell recruitment and CCD cell plasticity. Notch may play a role in all cases of cell plasticity in the adult kidney, through mechanisms retained from early development, and specific to each cell type, depending on their origin.[95]

5.2 | JG cells and other pericytes

While this review focuses on JG cells, other renal pericytes present characteristics of plasticity and may be key for homeostasis or tissue regeneration mechanisms. Pericytes play an important role in vessel development and maintenance, as well as a vast array of other specialized functions[96] and are characterized by their adaptability to their localization and the pathophysiological condition they are in. In the kidney, pericytes are situated both within, and adjacent to, glomeruli (mesangial[97] and JG cells) as well as around the peritubular capillaries where they possess contractile abilities.[98] However, pericytes are heterogeneous and, whilst their potential for plasticity is important, a detailed description of their plasticity was beyond the scope of this review.[99]

5.3 | Advances in technologies

To investigate time-sensitive phenomena, specific techniques such as scRNASeq, together with algorithms such as pseudotime or RNA velocity analysis are able to infer directionality to transcriptional activity between different cell types.[100] The latest developments in scRNASeq make it possible directly to link a cell's transcriptome to its location within a tissue (in situ scRNASeq, FISSEQ).[101,102] These techniques preserve spatial information about RNA location, a useful feature when dealing with cells such as principal and ICs of the collecting duct with inter-regulating phenotypes. Similarly, single cell proteomics and in situ single cell proteomics[103,104] will prove to be informative.

DNA editing technologies such as CRISPR-Cas9, with recent innovations including site-specific DNA insertion without homologous recombination,[105] and the Prime Editing "search-and-replace" technique,[106] will make the manipulation of factors involved in cell plasticity easier, allowing their use in healthy tissues for lineage tracing studies.

Imaging technologies need to be adapted for time-dependant phenomena. Intravital microscopy has been used to look at the dynamics of renal cell death[107] and could be used for other types of dynamic observation with adapted labelling.[108] Live-cell correlative light-electron microscopy (live-cell CLEM) offers the possibility of simultaneously recording the dynamics of subcellular components whilst imaging their structural properties.[109] Label-free imaging of live cells has been demonstrated using interferometric scattering microscopy,[110] showing intracellular organelles as well as topological characteristics of the membrane. Apart from new microscopy techniques, other live cell imaging techniques include new fluorescent probes[111,112] as well as improvements in reporters for visualization of cell signalling dynamics.[113]

A further technical development for observing cell plasticity is the use of in vitro models generated by 3D tissue

engineering or bio-printing. In vivo studies lead to observation of plasticity and related physiological effects; in vitro models offer the possibility for more detailed phenotyping (eg electrophysiology) and novel approaches such as spatially specific placement of cells to address the role of cell-cell contacts, and paracrine effects, to understand the mechanisms involved. All of the existing techniques for renal structure bio-printing use the "biomimicry" approach,[114] which aims to replicate in vitro the natural in vivo situation. 3D engineered biological structures are in theory more accessible for injection of drugs and solutes, imaging and inclusion of transgenic cells than animal models. With appropriate tagging of specific markers, it may be possible directly to see a cell changing its "identity", and further elucidate the process of cell plasticity. Recently 3D in vitro models of kidney proximal tubules, using human primary proximal tubular epithelial cells have been developed.[115-117]

These new single cell sequencing and proteomics techniques offer the possibility to precisely map cell-cell interactions in parallel to the investigation of single cell molecular mechanisms. Together with the use of rapid, precise, genetic modification techniques, new advances in cell imaging, and the application of bespoke microfluidic and 3D printed technology, these techniques will provide novel means to address the mechanisms underlying cell plasticity.

Several major areas of cell plasticity remain unclear and it is currently difficult to define the scale of the phenomenon, compared to other physiological mechanisms, for maintaining homeostasis or in the response to injury. It is possible that many cells possess the ability for plasticity but can only be observed in a few specific situations where it predominates over other mechanisms for maintaining homeostasis. The specific nature of cell types and locations makes it difficult to generalize mechanisms and currently plasticity is best investigated on a "case-by-case" basis using tailored approaches (eg the need to polarize cells for the study of collecting duct cells). Secondly, the environmental factors triggering cellular plasticity are still mostly unknown, and most likely different for every cell type. Just as important as the factors themselves might be understanding their thresholds, or the combinatorial effects required to activate plasticity rather than an alternative type of cellular response.

6 | CONCLUDING REMARKS

Cell plasticity in adult tissue is a well-recognized phenomenon and has a role in maintaining homeostasis in healthy and disease states. Cells of the kidney have the ability to switch cell type depending on environmental cues and do so in a localization and cell type-specific manner. Cell plasticity in adult tissues is of great interest for regenerative medicine in nephrology.[118] These recent discoveries about cell plasticity in the glomerulus and tubules indicate that kidney has a constitutive ability to repair; though that ability is overcome by deleterious mechanisms in long-term diseases. A new approach—finding a way to keep and protect the cell regenerative/plasticity functions during CKD—could make a major contribution and presents alternative solutions to the use of outsourced stem cells for organ regeneration and repair.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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